19. The modified wild strain of enteroinvasive *Shigella* of claim 18, wherein the first gene comprises the ent F, Fep E, Fep C, and Fep D subunit genes of the enterochelin operon of the *S. dysenteriae* 1.

20. The modified wild strain of enteroinvasive *Shigella* of claim 17, wherein the *Shiga*-toxin gene is *Shiga*-toxin A.

21. The modified wild strain of enteroinvasive *Shigella* of claim 17, wherein one or more of the genes are inactivated by allelic exchange with one or more mutagenized genes, wherein said mutagenized genes have been mutagenized *in vitro* .

22. The modified wild strain of enteroinvasive *Shigella* of claim 21, wherein said mutagenized genes are mutagenized genes from which nucleotide sequences have been deleted.

23. The modified wild strain of enteroinvasive *Shigella* of claim 21, wherein said mutagenized genes are mutagenized genes into which marker genes have been inserted.--

REMARKS

Reconsideration of the application is respectfully requested. Claim 9 has been canceled. Claims 2-4, 6-8, 10, and 13 have been amended. Claims 15-24 are new. Support for the amendments is found throughout the specification, particularly on page 5, lines 18-28; page 11, paragraph 1; and page 21, pargraphs 3 and 4. New claims 15 and 16 find support in original claim 10. New claims 17-24 are drawn to the product of the method of claims 1-8 and 10.

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Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 1-10, 13, and 14 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to adequately teach one skilled in the art how to make and/or use the claimed invention. The Examiner argues that the construction of claimed *Shigella* mutants requires knowledge of the nucleotide sequence of said genes, which regions are responsible for biological activity, and the number of nucleotides which must be deleted or inserted. The Examiner alleges that due to the limited teaching of the specification and the unpredictable nature of which mutations are useful one skilled in the art can not practice the invention as claimed absent undue experimentation.

The Examiner further asserts that while it would appear that techniques are known in the art for inactivation of genes, it is not routine to screen for positions within the DNA sequence of the gene so that it does not invade the cells, does not spread within infected cells, or does not produce toxins. The Examiner contends that the specification does not disclose which regions of the genes are responsible for biological activity, the number of nucleotides which must be deleted or inserted, the identity of the genes responsible for invading cells, and the identity of genes that code for aerobactin and enterochelin, that more than one gene would be expected to be involved in toxin production, spreading, and/or invasion, and that the specification gives no guidance as to which of the essentially infinite choices would be successful. The Examiner concludes that modifications that can be made to inactivate genes is unpredictable, and that

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undue experimentation would be required to practice the claimed invention. Applicants respectfully traverse this rejection.

1. **The claimed method does not require knowledge of nucleotide sequence of target genes**

Contrary to the assertion of the Examiner, the mutagenesis technique taught by the specification does not require knowledge of the nucleotide sequence of the target genes. Prentki and Krisch, 1984 (Exhibit 1), disclose a method of *in vitro* insertional mutagenesis used by the applicants in embodiments of the claimed invention. (Specification at 9-23.)

The technique disclosed in Prentki and Krisch allows mutagenesis by insertion of a selectable marker (interposon) at any restriction site in a plasmid (Prentki and Krisch, Figure 5). The target plasmid and the interposon are linearized by restriction enzyme digestion. Id. at 307. Linearization of plasmid DNA containing many recognition sites is performed in the presence of ethidium bromide. Id. at 304. This allows full size linear plasmids to be obtained. Id. at 309. The cut ends of these DNA can be blunted by treatment with DNA polymerase. Id. at 307. The DNAs are blunt-end ligated, and recombinant bacteria expressing the selectable marker are selected. Id. at 307. The insertion of the interposon abolishes mRNA synthesis and interrupts protein synthesis. Id. at 307. No sequence information of the target gene was required to obtain the desired mutants in Prentki and Krisch. Therefore, applicants submit that sequence information of target genes is not necessary for the successful implementation of the claimed invention.

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2. The claimed method does not require knowledge of regions of genes responsible for biological activity

Contrary to the assertion of the Examiner, knowledge of the regions of genes responsible for biological activity is not necessary for the successful implementation of the claimed invention. The insertion of an interposon by the method of Prentki and Krisch introduces both transcriptional and translational stop signals. Id. at 303. Therefore, the selectable insertion of the interposon inactivates both RNA and protein synthesis downstream of the site of insertion. Id. at 312. All insertions of the interposon into the LacZ gene of plasmid placB235 totally abolished B-galactosidase activity. Id. at 309 and Table 1. Therefore, the skilled artisan expects success in activating a target gene, using the technique of Prentki and Krisch.

No knowledge of the regions of genes responsible for biological activity was required in Prentki and Krisch. Therefore, applicants submit that knowledge of the regions of genes responsible for biological activity is not necessary for the successful implementation of the claimed invention. Furthermore, using the claimed invention, the skilled artisan expects success in inactivating the target gene, absent evidence to the contrary.

3. The number of nucleotides deleted or inserted is not critical to the practice of the claimed invention

Contrary to the assertion of the Examiner, the number of nucleotides deleted or inserted is not critical to claimed invention. Prentki and Krisch indicate that the most important aspect of the mutagenesis procedure is the selectable introduction of translational and transcriptional stop

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signals. Id. at 312. The selectable marker can be subsequently removed by restriction enzyme digestion and ligation, indicating that the size of the insertion is not critical to the practice of this technique. Id., Figure 5. The specification teaches the use of other selectable markers within the interposon, for example mercury, arsenate, arsenite, antimony, cadmium, zinc, and cobalt resistance. (Specification at 6, paragraph 1.) The skilled artisan recognizes that the size of these insertions will be variable, and that size is not critical to the practice of the claimed invention.

It is also evident from Prentki and Krisch that the deletion of a specified number of nucleotides is not critical for the practice of the claimed invention. The specification teaches that the genes are wholly or partly removed or permanently inactivated, preferably at least partly removed. (Specification at 5, paragraph 2.) As an example, the specification teaches that a 400 base pair deletion can be made using *Bal*31, starting at a *Hpa*I restriction site. (Id. at 22, paragraph 2.) Furthermore, the specification teaches that the mutagenized genes have had a significant portion of the genes deleted. (Id. at 6, paragraph 1.) This is to prevent loss of the inactivated phenotype in subsequent *Shigella* generations. (Id. at 6, paragraph 1.) The skilled artisan recognizes that the number of nucleotides that are removed is not critical, but that the ability to lose the inactivated phenotype will be reduced by a significant deletion. (See Id. at 6, paragraph 1.) The skilled artisan, using the technique of Prentki and Krisch and the teachings of the specification, would merely make a significant deletion at the restriction site prior to the insertion of the interposon. Therefore, applicants submit that neither the precise number of

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nucleotides inserted nor the precise number of nucleotides deleted are critical to the practice of the invention.

4. <u>The prior art teaches methods for the determination of and the genetic regions involved in invasion and spread of *Shigella*</u>

Applicants previously submitted prior art references that indicated the level of skill in the art prior to the filing of the instant application. These included Nassif et al. 1987, which discloses assay procedures for screening for *Shigella* mutations that affect invasion of cells, toxin production, and spread; Baudry et al., 1987, which discloses assay procedures for screening for *Shigella* mutations that affect invasion of cells and toxin production; and Maurelli et al., which discloses assay procedures for screening for *Shigella* mutations that affect invasion of cells, toxin production, and spread. Baudry et al., 1987, also discloses a genetic restriction map of a portion of the *Shigella* invasion plasmid that defines genetic regions involved in the invasion phenotype (Figure 1).

The Examiner dismissed Baudry et al. because the reference indicates that invasion is a complex process that involves many genes and polypeptides. The Examiner dismissed Nassif et al. as only being directed to one gene that codes for aerobactin. The Examiner did not address the teachings of Maurelli et al.

Applicants respectfully submit that the Examiner is applying an improper test of enablement. The test for enablement is whether one skilled in the art could make or use the invention from the disclosures in the patent *coupled with information known in the art* without

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undue experimentation. (M.P.E.P. § 2164.01; italics added.) Although some experimentation may be necessary to carry out the claimed invention, the experimentation would not be undue, but merely routine.

Applicants submit herewith additional references indicative of the skill in the art at the time the application was filed. Hale et al., 1983 (Exhibit 2), discloses assay procedures for screening for *Shigella* invasion of cells (page 341), and that *Shigella* plasmids conferred the invasive phenotype (page 346). Sasakawa et al., 1986 (Exhibit 3), discloses assay procedures for screening for *Shigella* mutations that affect invasion of cells and spread. Sasakawa et al. at 33. Sasakawa et al. further discloses the location and inactivation of virulence determinants within the invasion plasmid of *Shigella* and a restriction map of the invasion plasmid. Id. at Table 2 and Figure 3. Sasakawa et al., 1988 (Exhibit 4), further defines the genetic regions associated with invasion and discloses a more detailed restriction map of the *Shigella* invasion plasmid (Figure 1). Maurelli et al., 1985 (Exhibit 5), discloses assay procedures for screening for *Shigella* mutations that affect invasion of cells (page 165), as well as a restriction map of recombinant plasmids derived from the *Shigella* invasion plasmid, which were capable of conferring the invasive phenotype (Figure 4). Venkatessen et al., 1988 (Exhibit 6), also discloses a genetic restriction map of a portion of the *Shigella* invasion plasmid (Figure 2). In addition, Buysse et al., 1987 (Exhibit 7), and Sakai et al., 1986 (Exhibit 8), disclose genetic restriction maps of a portion of the *Shigella* invasion plasmid (Figure 5 and Figure 1, respectively). Sakai et al. also

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discloses the nucleotide sequence of *vir*F (Figure 2). Baudry et al., 1988 (Exhibit 9), discloses the nucleotide sequence of a portion of the *Shigella* invasion plasmid (Figure 3).

Applicants also submit herewith addition references Herrero et al., 1988 (Exhibit 10); Bindereif and Neilands, 1983 (Exhibit 11); Lawlor and Payne, 1984 (Exhibit 12); Lundrigan and Kadner, 1986 (Exhibit 13); Pettis and McIntosh, 1987 (Exhibit 14); and Gross et al., 1985 (Exhibit 15). These references indicate the state of the art at the time the application was filed with respect to knowledge of aerobactin and enterochelin synthesis. These references describe the genetic and molecular characterization of genes involved in aerobactin and enterochelin synthesis.

Applicants submit that, using the disclosure of these references in combination with the teachings of the specification, the skilled artisan could practice the claimed invention, absent evidence to the contrary. The enablement requirement of U.S.C. § 112 does not require applicants to disclose every species covered by a claim. *In re Angstadt*, 37 F.2d 498, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976).

Applicants submit that the prior art discloses a wealth of information concerning the genes involved in the spread and invasion of *Shigella*. Applicants further submit that the prior art teaches the requisite screening procedures for screening for mutations in *Shigella* genes that affect the invasion of cells, spread within infected cells, and toxin production. Applicants submit that genetic targets and techniques for such screening were known to the skilled artisan and, in combination with the guidance of the specification, enable the skilled artisan to make and use the

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claimed invention. In the absence of any evidence of non-enablement, applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

Double-Patenting Rejection

The Examiner maintains the provisional rejection of claim 13 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over copending application Ser. No. 08/118,100.

Applicants have amended claim 13 to recite that the *Shigella* is other than that designated SC501, SC504, SC505, and SC506. Accordingly, applicants submit that claim 13 and the claims of application Ser. No. 08/118,100 are patentably distinct, and respectfuly request withdrawal of the rejection.

Claim Objection

Claims 1-10 were objected to for the phrase "uninfected, cells" in claim 1.

Applicants traverse the objection. Applicants submit that the full recitation in claim 1 of "infected, as well as uninfected, cells" is an appropriate phrase, and respectfully request withdrawal of the objection.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 2-10 were rejected under 35 U.S.C. § 112, second paragraph, for allegedly failing to point out and distinctly claim the invention.

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Claims 4 and 7 were rejected for being allegedly vague and indefinite in the use of the term "intra-intercellular spread." Claims 4 and 7 have amended to recite "intra- or intercellular spread." Accordingly, applicants respectfully request withdrawal of the rejection.

Claim 8 was rejected for being allegedly vague and indefinite in the use of the phrase "the first gene comprises the . . . genes." Applicants have amended claims 2, 3, 6, and 8 to recite "gene or genes." Accordingly, applicants respectfully request withdrawal of the rejection.

Claim 10 was rejected for being allegedly vague and indefinite in the use of the terms "*in vitro* mutagenized genes" and "significant portions." Applicants have amended claim 10 to clarify that the mutagenized genes are mutagenized *in vitro*. Applicants have rewritten claim 10 to delete the recitation of "significant portions" and have added claims 15 and 16 to further clarify the claimed invention. Accordingly, applicants respectfully request withdrawal of the rejection.

Claim 2 and dependent claims were rejected because it is allegedly unclear what is "wholly or partly removed or permanently inactivated." Applicants have amended claim 2 to more clearly indicate that both genes are wholly or partly removed or permanently inactivated. Accordingly, applicants respectfully request withdrawal of the rejection.

The Examiner further contends that claim 2 is vague and indefinite because a gene that is wholly or partly removed is permanently inactivated. As indicated above, a gene can be permanently inactivated without being wholly or partly removed, for instance by insertion of a selectable marker. Accordingly, applicants respectfully request withdrawal of the rejection.

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Rejection Under 35 U.S.C. § 112, Fourth Paragraph

Claim 9 was rejected under 35 U.S.C. § 112, fourth paragraph, as being of improper dependent form for allegedly failing to limit the subject matter of claim 5.

Applicants have canceled claim 9. Accordingly the rejection is moot.

Objection to the Specification

The specification was objected to for allegedly not containing reference to the status of the prior applications. Applicants have amended the specification to add the requested reference. Accordingly, applicants respectfully request withdrawal of the objection.

Conclusions

In view of the foregoing remarks, applicants believe that this application is now in condition for allowance. If the Examiner should disagree, he is invited to contact the undersigned to discuss any remaining issues.

To the extent any extension of time under 37 C.F.R. § 1.136 is required to obtain entry of this paper, such extension is hereby requested. If there are any fees due under 37 C.F.R. §§ 1.16

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or 1.17 which are not enclosed, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge those fees to our Deposit Account No. 06-916.

Respectfully submitted,

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By: _____
Kenneth J. Meyers
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Dated: April 17, 1998

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